PHARMACOLOGY AND PHARMACY

NEUROPROTECTIVE EFFECT OF *OROSTACHYS SPINOSE* DRY EXTRACT IN CHOLINERGIC INSUFFICIENCY

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ABSTRACT

Background. *Orostachys spinosa (L.) Sweet. – a perennial plant of a wide habitat and contains various metabolites (amino acids, flavonoids, polysaccharides, etc.). Extracts from the aerial part of the plant are used in traditional medicine as an anticonvulsant and sedative.*
The aim of the work. *To study the neuroprotective effect of O. spinosa in cholinergic deficiency.*
Materials and methods. *The studies were carried out on 52 Wistar rats. The animals were administered scopolamine (1 mg/kg) daily for 21 days, followed by O. spinosa dry extract per os at a dose of 100 mg/kg for 14 days. On the day 32, the animals developed a conditioned passive avoidance reflex (CPAR), the integrity of which was checked after 1, 24 and 72 hours; on the day 35 they were tested in an "open field". On the day 36, biochemical and histological studies of the brain were carried out.*
Results. *It has been established that O. spinosa, against the background of scopolamine intoxication, reduces the anxiety of animals, stimulates exploratory activity in the open field test, improves the production and preservation of the CPAR, and also reduces the number of functionally inactive neurons (pyknotic and shadow cells) in the cerebral cortex. The extract reduces the lactate/pyruvate ratio by 47 %, intensifies the activity of mitochondrial complexes I and II by 54–64 %, and increases the concentration of adenosine triphosphate by 1.6 times compared to the control. O. spinosa exhibits antioxidant properties by reducing malondialdehyde and increasing the activity of catalase, glutathione peroxidase and glutathione reductase in the brain.*
Conclusion. *O. spinosa dry extract has a neuroprotective effect in cholinergic deficiency. The studied extract exhibits antioxidant properties and stimulates energy processes in the brain.*

Key words: *Orostachys spinosa (L.) Sweet, dry extract, neuroprotective effect, scopolamine hydrochloride, cholinergic insufficiency*

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ИССЛЕДОВАНИЕ НЕЙРОПРОТЕКТИВНЫХ СВОЙСТВ *OROSTACHYS SPINOSA* ЭКСТРАКТА СУХОГО ПРИ ХОЛИНЕРГИЧЕСКОЙ НЕДОСТАТОЧНОСТИ

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РЕЗЮМЕ

Обоснование. *Orostachys spinosa (L.) Sweet – многолетнее растение, имеющее широкий ареал произрастания и содержащее различные метаболиты (аминокислоты, флавоноиды, полисахариды и др.). Извлечения из надземной части растения используются в традиционной медицине в качестве противосудорожного и седативного средства.*

Цель работы. *Исследовать нейропротективные свойства O. spinosa при холинергической недостаточности.*

Материалы и методы. *Исследования проведены на 52 крысах линии Wistar. Животным ежедневно в течение 21 дня вводили скополамин (1 мг/кг), далее в течение 14 дней – per os O. spinosa экстракт сухой в дозе 100 мг/кг. На 32-е сутки у животных вырабатывали условный рефлекс пассивного избегания (УРПИ), сохранность которого проверяли через 1, 24 и 72 часа; на 35-е сутки тестировали в «открытом поле». На 36-е сутки проводили биохимические и гистологические исследования головного мозга.*

Результаты. *Установлено, что O. spinosa на фоне скополаминовой интоксикации снижает тревожность животных, стимулирует исследовательскую активность в «открытом поле», улучшает выработку и сохранность УРПИ, а также снижает количество функционально неактивных нейронов (пикнотических и «клеток-теней») в коре больших полушарий головного мозга. Экстракт снижает соотношение лактат/пируват на 47 %, интенсифицирует активность митохондриальных комплексов I и II на 54–64 %, увеличивает концентрацию аденозинтрифосфата в 1,6 раза по сравнению с контролем. O. spinosa проявляет антиоксидантные свойства, снижая содержание малонового диальдегида, повышая активность каталазы, глутатионпероксидазы и глутатионредуктазы в головном мозге.*

Заключение. *O. spinosa экстракт сухой оказывает нейропротективное действие при холинергическом дефиците. Исследуемый экстракт проявляет антиоксидантные свойства и стимулирует энергетические процессы в головном мозге.*

Ключевые слова: *Orostachys spinosa (L.) Sweet, экстракт сухой, нейропротективное действие, скополамин гидрохлорид, холинергическая недостаточность*

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According to the World Health Organization, the world is experiencing demographic aging of the population. As a result, the number of cerebral and neurodegenerative diseases is increasing, and dementia and depression are among the most common diseases of the elderly [1]. All this determines the need to develop new approaches to finding rational treatment and prevention of the nervous system diseases. In the prevention of neurological diseases, herbal medicines deserve attention, which are capable of having a polymodal effect on the body due to a significant variety of metabolites [2].

In this regard, plants of the genus *Orostachys* are of interest. Thus, extracts of *O. japonicas*, used in Japanese and Korean traditional medicine as an adaptogenic agent, have an inhibitory effect on acetylcholinesterase [3], exhibit an antioxidant effect [4], and also limit neuronal apoptosis [5]. Another perennial plant of the Crassulaceae family, *Orostachys spinosa* (L.) Sweet, has similar properties. It is used in folk and traditional medicine of different peoples for diseases of the gastrointestinal tract, respiratory and nervous systems [6]. The main active compounds of *O. spinosa* are amino acids, flavonoids, coumarins, polysaccharides, fatty acids, etc. [7]. According to experimental studies, a liquid extract from the herb *O. spinosa* exhibits anxiolytic, nootropic, antihypoxic and stress-protective properties [8–10]. A dry extract was obtained from the above-ground part of *O. spinosa*, which is characterized by a constant composition [11], exhibiting neuroprotective properties in experimental cerebral ischemia [12]. In this regard, it is of interest to evaluate the neuroprotective properties of *O. spinosa* dry extract in modeling neurodegenerative disease.

THE AIM OF THE STUDY

To study the neuroprotective effect of *Orostachys spinosa* dry extract in cholinergic deficiency.

MATERIALS AND METHODS

The studies were performed on 52 Wistar rats weighing 200–220 g. The animals were kept in accordance with the Good Laboratory Practice (GLP) and the Resolution of the Russian Government No. 855 dated June 13, 2020. The research work was carried out in accordance with the Rules adopted in the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). The study protocol was approved by the Ethics Committee of the Institute of General and Experimental Biology of the Siberian Branch of the Russian Academy of Sciences (No. 2 dated December 1, 2020).

The object of the study was a dry extract obtained from the above-ground part of *O. spinosa* by successive three-fold extraction of crushed raw materials with 10 % ethyl alcohol at a temperature of 60 °C, followed by filtration, evaporation and vacuum drying [8]. The extract was standardized by the content of the free amino acids sum in terms of glutamic acid, which should be at least 3.0 %.

Ginkgo biloba leaf extract (tanakan, tablets; Beaufour Ipsen Industrie, France) was used as a comparison drug. The action of *G. biloba* preparations is based on antioxidant properties and the ability to normalize neurotransmitter and energy processes in the brain [13].

To model chronic cholinergic deficiency, animals in the control and experimental groups were administered scopolamine hydrochloride intraperitoneally at a dose of 1 mg/kg for 21 days [14]. Scopolamine hydrochloride is considered a non-selective muscarinic receptor antagonist that causes cognitive impairment and electrophysiological changes in the brain similar to those seen in natural aging and Alzheimer's disease [15]. Scopolamine also causes a number of cellular changes, including antioxidant defense system disruption, increased oxidative stress, mitochondrial dysfunction, apoptosis, and neuroinflammation [16].

The animals were divided into four groups. The first experimental group consisted of rats (n = 13), which after a 21-day scopolamine injection were administered intragastrically once a day for 14 days *O. Spinose* dry extract at a dose of 100 mg/kg in the form of an aqueous solution in a volume of 10 ml/kg. According to a similar scheme, the animals of the second experimental group (n = 13) were administered *G. biloba* extract at a dose of 100 mg/kg, and the animals of the control group (n = 12) were administered water in a volume of 10 ml/kg. In intact control animals (n = 14), cholinergic insufficiency was not modeled; they were administered physiological saline intraperitoneally for 21 days, then they were administered water intragastrically in a volume of 10 ml/kg for 14 days.

On the 32nd day, the animals were trained in the conditioned passive avoidance reflex (CPAR) test [14]; the conditioned reflex was tested after 1, 24, and 72 hours. On the 35th day, the animals were tested in the open field [14]. On the 36th day after the start of test extract administration, the animals were decapitated under ether anesthesia, and the brain was removed for biochemical and histological studies.

The intensity of lipid peroxidation processes was determined by the content of malondialdehyde (MDA) [17]; the state of the antioxidant system – by the activity of catalase (CAT) [18], glutathione peroxidase (GPO) and glutathione reductase (GR) [19]. The effect of the studied agent on energy processes in the brain was assessed by the content of adenosine triphosphate (ATP) [20], the activity of NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II) [21, 22]. The glycolysis intensity was characterized by the activity of pyruvate kinase (PK) [23] and the content of lactate and pyruvate in the brain homogenate [20].

Brain sections for histological examination were prepared using a standard technique on an MS-2 microtome (SPECTRO LAB, Russia), then stained with cresyl violet according to Nissl [24]. In layers II–V of the brain frontal cortex, the number of neurons was counted, which were differentiated into normochromic, intensely hypochromic, intensely hyperchromic (pyknotic), and "shadow cells".

Statistical data processing was performed using Statistica for Windows 6.0 (StatSoft Inc., USA). The conformity of the analyzed features to the normal distribution law was assessed using the Shapiro – Wilk criterion. The statistical significance of differences between the data of the groups was determined using Student's *t*-test, provided that the sample has a normal distribution; using the Mann – Whitney criterion if the data do not obey the normal probability distribution. The data are presented as the arithmetic mean (M) and the arithmetic mean error (m). To compare the number of animals in the comparison groups, the Fisher's angular transformation φ-test was used. Differences between the experimental and control groups were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of testing animals in the "open field" showed that scopolamine inhibits motor and exploratory activity in animals (fig. 1), which is reduced by the *O. spinosa* extract administration. Thus, in animals that were administered *O. spinosa* extract, the number of entries into the installation central squares increased by 2.1 times, vertical stands and peeks into the "holes" – on average 1.7 times compared to the control values. At the same time, in animals of the 2nd experimental group, the number of central squares and the hole reflex were higher than in the control, by 1.6 and 1.3 times, respectively. Against the background of long-term scopolamine administration in animals of the 1st experimental group, the number of grooming acts decreased by 1.6 times compared to the control value (fig. 1).

When *O. spinosa* extract was administered, CPAR was formed in 100 % of animals and was retained after 24 and 72 hours in 92 and 77 % of animals, respectively (fig. 2). In the 2nd experimental group, the conditioned reflex was formed in the same way as in the control group, in 75 % of animals, and by the 3rd day it was retained in all of these animals, while in the control group it was retained only in 50 %.

It was found that multiple scopolamine injections lead to a reduction in the energy potential of brain cells, which is associated with a decrease in the intensity of anaerobic and aerobic processes (fig. 3, 4). According to the data presented in Figure 3, PK activity in the brain homogenate of control animals decreased by 25 %, and the pyruvate content – by 22 % compared to the intact

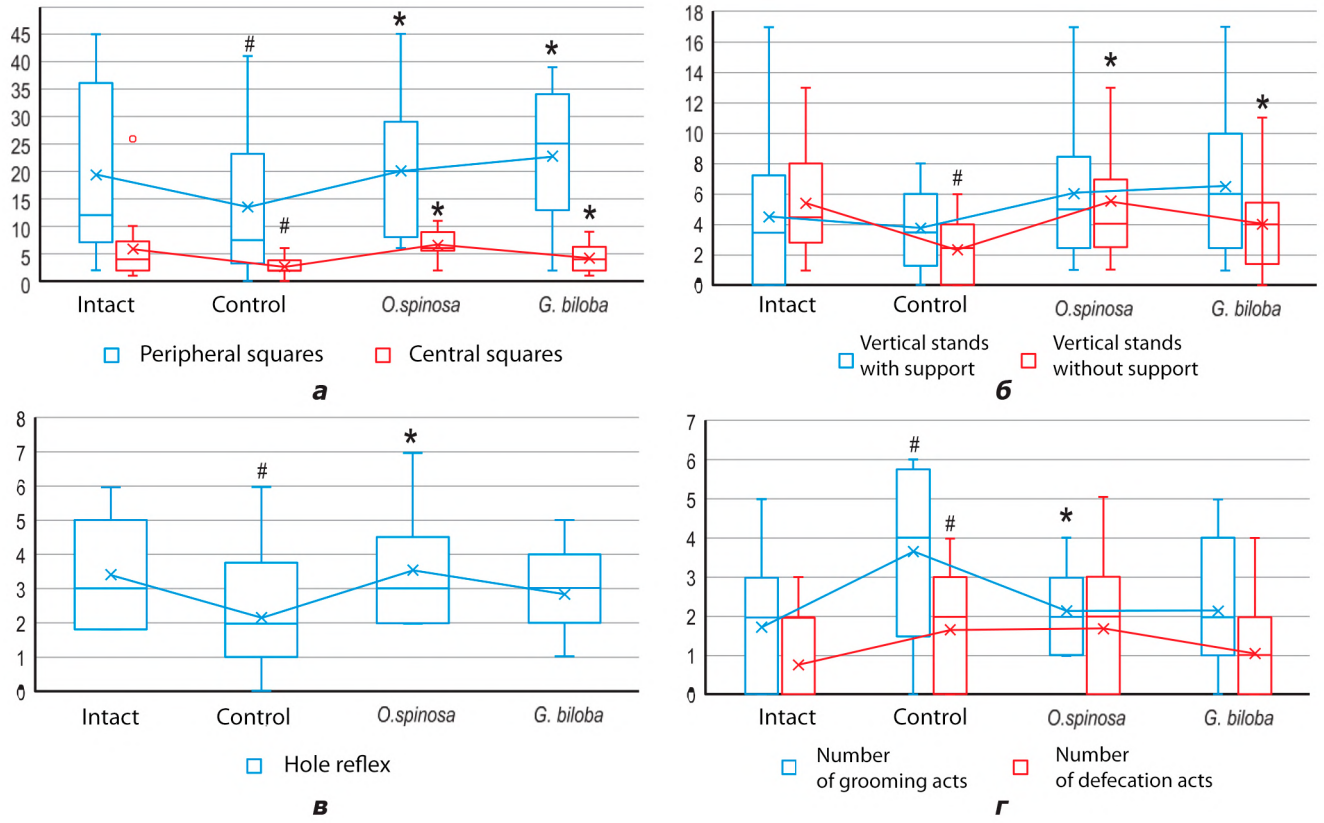


FIG. 1.
*Behavioral indicators of Wistar rats in the open field test in cholinergic insufficiency: **а** – horizontal activity; **б** – vertical activity; **в** – hole reflex; **г** – anxiety indicators. Statistical significance of differences was determined using Mann – Whitney test: # – the differences are statistically significant compared to the indicators of the intact group at $p \leq 0.05$; * – the differences are statistically significant compared to the indicators of the control group at $p \leq 0.05$*

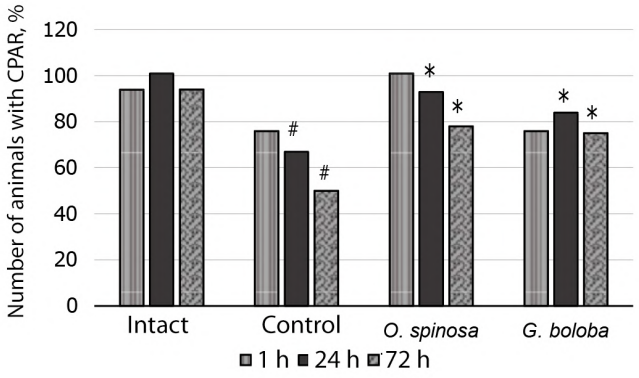


FIG. 2.
*Number of Wistar rats with a conditioned passive avoidance reflex in cholinergic insufficiency. Statistical significance of differences was determined using Fisher's φ test: # – the differences are statistically significant compared to the indicators of the intact group at p ≤ 0.05; * – the differences are statistically significant compared to the indicators of the control group at p ≤ 0.05*

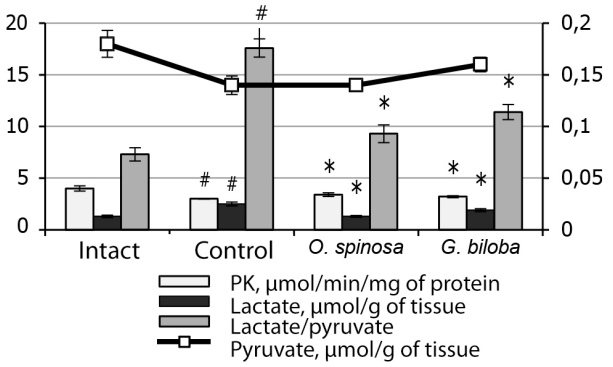


FIG. 3.
*Glycolysis indicators in the Wistar rats brain in cholinergic insufficiency. Data are presented as arithmetic mean (M) and arithmetic mean error (m). Statistical significance of differences was determined using Student's t-test: # – the differences are statistically significant compared to the indicators of the intact group at p ≤ 0.05; * – the differences are statistically significant compared to the indicators of the control group at p ≤ 0.05*

control animals. Against the background of a decrease in pyruvate concentration, the lactate level in the brain of control animals was 2.5 ± 0.19 μmol/g of tissue, which is 42 % higher than the intact value. As a result, the lactate/pyruvate ratio in the control increased by 2.4 times, reaching 17.6 ± 0.88 (fig. 3). Also, in the control, the activities of mitochondrial complexes I and II decreased twofold, as a result, the ATP concentration in the brain was 0.7 ± 0.08 μmol/g tissue, which is 2.4 times lower than the intact value (fig. 4).

Against the background of the *O. Spinose* dry extract use, the PK activity increased by only 13 %, and the pyruvate content almost corresponded to that in the control animals (fig. 3). At the same time, the lactate level in animals of the 1st experimental group was 48 % lower than the control value and corresponded to that in animals of the intact control. As a result, the lactate/pyruvate ratio in animals of this experimental group was 47 % lower than in the control, while in the 2nd experimental group it was 35 %. The activities of mitochondrial complexes I and II in rats administered with *O. spinosa* extract were higher by 57 and 64 %, and *G. biloba* extract by 87 and 20 %, respectively, than in the control (fig. 4). As a result, the ATP content in the brain of animals of the experimental groups increased on average by 1.6 times compared to the control value.

Energy metabolism disorders that develop with cholinergic insufficiency contribute to increased production of free radicals, as well as inhibition of the activity of antioxidant enzymes [16], which leads to irreversible processes and neuronal death. Thus, in animals of the control group, against the background of an increase in the MDA content (by 2.2 times), a decrease in the activity of antioxidant enzymes is observed: CAT by 42 %, GPO by 67 % and GR by 52 %, relative to the indicators in intact control animals (table 1).

Amid the administration of extracts, a decrease in the MDA concentration in the brain homogenate by 25–30 % was noted compared to the control indicator. The activity of enzymes – CAT, GPO and GR – in the brain tissue of animals of the 1st experimental group increased by 36, 78 and 32 %, the 2nd experimental group – by 67, 68 and 34 %, respectively, compared to those in the control animals.

Pathomorphological studies of the cerebral cortex showed that against the background of long-term scopolamine hydrochloride administration, most neurons in layers II–V decreased in size, the nuclei and cytoplasm appeared uniformly stained, the apical dendrite became thinner and could be traced over a long distance, "spirally twisting" (fig. 6a). On average, the number of intensely hyperchromic neurons in layers II–V was 77 % greater than the intact indicator, amounting to 10.6 ± 1.35 % of the total number of neurons (fig. 5). A greater number of "shadow cells" (by 48 %) were also observed; these neurons showed karyolysis amid homogeneous cytoplasm due to chromatolysis. Most "shadow cells" were subject to satellitosis and neuronophagy.

In animals that received the extracts under study, the pathomorphological picture of the cerebral cortex did not look as "mosaic" as in animals of the control group. Pyknotic neurons were detected singly, in most cases only in layers III and V (fig. 6 б, в). Their number was on average 2.0 times lower than in the control. "Shadow cells" and the accompanying processes of satellitosis and neuronophagy were observed much less frequently, but in all cerebral cortex layers. Due to the decrease in the total number of regressive neurons, the number of normochromic cells in animals of the 1st experimental group was 11 % higher than in the control.

Thus, *O. spinosa* dry extract against the background of long-term cholinergic insufficiency has

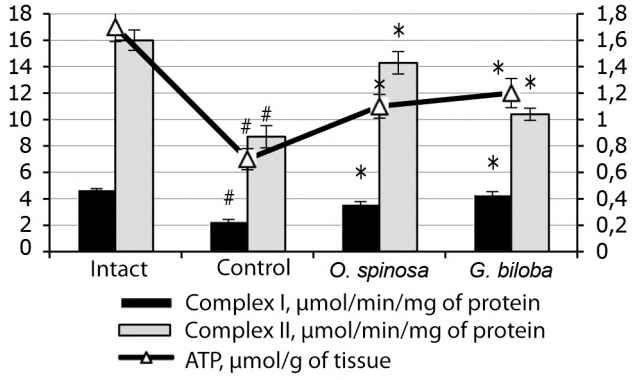


FIG. 4.
*Activity of mitochondrial complexes I and II and ATP content in the Wistar rats brain in cholinergic insufficiency. Data are presented as arithmetic mean (M) and arithmetic mean error (m). Statistical significance of differences was determined using Student's t-test: # – the differences are statistically significant compared to the indicators of the intact group at p ≤ 0.05; * – the differences are statistically significant compared to the indicators of the control group at p ≤ 0.05*

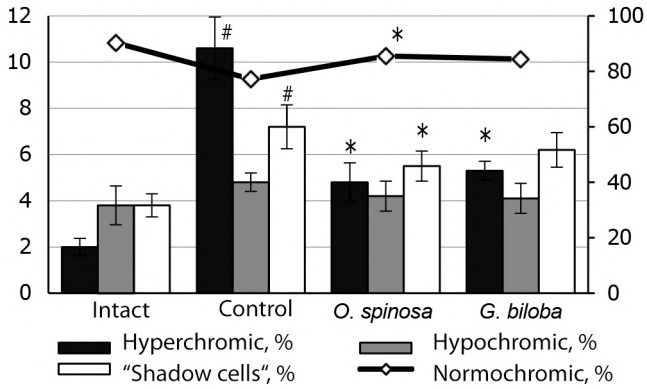


FIG. 5.
*Morphometric parameters of neurons in the cerebral cortex of Wistar rats in cholinergic insufficiency. Data are presented as arithmetic mean (M) and arithmetic mean error (m). Statistical significance of differences was determined using Student's t-test: # – the differences are statistically significant compared to the indicators of the intact group at p ≤ 0.05; * – the differences are statistically significant compared to the indicators of the control group at p ≤ 0.05*

an antiamnestic effect and normalizes the emotional state of animals; in particular, it improves the formation and preservation of conditioned reflexes, and stimulates orientation-exploratory activity. *O. spinosa* extract increases the brain resistance to the toxic effect of scopolamine, limiting the number of regressive forms of neurons (hyperchromic and "shadow cells") and increasing the number of functional neurons in the cerebral cortex. Neuroprotective effect of *O. spinose* extract is associated with its ability to influence the functional activity of NADH dehydrogenase and succinate dehydrogenase complexes of the mitochondrial respiratory chain, correct the processes of aerobic glycolysis, reduce the content of MDA products, increase the intensity of the endogenous antioxidant system by increasing the activity of enzymes (GPO, GR and CAT) in the brain.

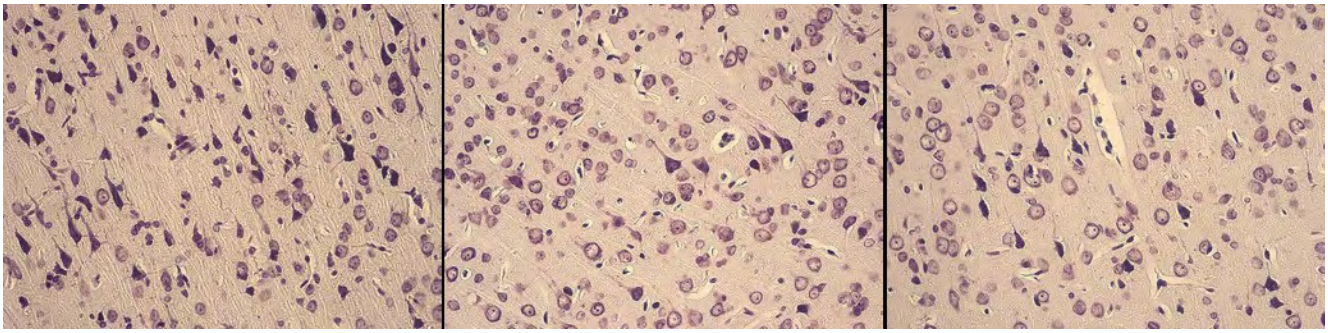
The identified neuroprotective effect of the *O. spinosa* dry extract is due to the presence of various compounds, among which flavonoids, amino acids, polysaccharides and coumarins predominate. *O. spinosa* metabolites help to inhibit dysfunction of the cholinergic system and oxidative stress. Thus, myricetin showed pronounced neuroprotective activity in the scopolamine model of Alzheimer's [25]. This flavonoid exhibits an antiamnestic effect in conditions of cognitive impairment caused by chronic stress [26], as well as amid the administration of streptozotocin [27] and D-galactose [28]. The flavonoid luteolin identified in *O. spinosa* exhibits a neuroprotective effect. Luteolin reduces cognitive dysfunction in rats with Alzheimer's disease by removing oxygen free radicals, increasing antioxidant potential, reducing NF-κB and BACE1 expression, and decreasing Aβ deposition [29]. A certain contribution to the neuroprotective effect of the studied extract is made

TABLE 1

INDICATORS OF THE PRO- AND ANTIOXIDANT SYSTEM OF THE WISTAR RATS BRAIN IN CHOLINERGIC INSUFFICIENCY, M ± M

Group	MDA, μmol/g of tissue	CAT, μmol/min/g of tissue	GPO, nmol/min/mg of protein	GR, nmol/min/mg of protein
Intact	8.4 ± 0.55	11.0 ± 0.70	49.2 ± 2.50	63.1 ± 6.36
Control	18.7 ± 0.97#	6.4 ± 0.12#	16.4 ± 1.10#	30.2 ± 2.01#
O. spinosa	14.0 ± 1.15*	8.7 ± 0.34*	29.2 ± 1.78*	40.0 ± 1.11*
G. biloba	13.0 ± 1.19*	10.7 ± 0.30*	27.5 ± 2.10*	40.4 ± 2.40

Note. The statistical significance of the differences was determined using Student's t-test: # – differences are statistically significant relative to the indicators of the intact group at $p \leq 0.05$; * – differences are statistically significant relative to the indicators of the control group at $p \leq 0.05$.



а *б* *в*

FIG. 6.
Microphotographs of Wistar rats cerebral cortex in long-term cholinergic insufficiency (Nissl cresyl violet staining, magnification ×200): ***а*** *– control;* ***б*** *– O. spinose;* ***в*** *– G. biloba*

by coumarins, which, due to their antiplatelet and anticoagulant properties, help to normalize cerebral blood flow. The neuroprotective effect of coumarins is also realized by inhibiting oxidative stress and neuroinflammation [30]. Amino acids and polysaccharides contained in the *O. spinosa* extract also have pharmacotherapeutic efficacy in the treatment of nervous system diseases through various mechanisms, including inhibition of oxidative stress, neuroinflammation, cellular apoptosis, and excitotoxicity [31, 32].

CONCLUSION

Thus, the *O. spinosa* dry extract exhibits neuroprotective properties with long-term scopolamine administration, preventing the development of "anxiety" in animals, improving learning and memory processes amid limiting changes in the cerebral cortex neurons. The neuroprotective effect of the *O. spinosa* dry extract is associated with its ability to stimulate the antioxidant system activity and metabolic processes in the brain.

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Conflicts of interest

No apparent and potential conflicts of interests relevant to this article reported.

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